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Development of the *Spirillum volutans* Motility Test for Toxicity Studies

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Research Management Division
14th Floor, Standard Life Centre
10405 - Jasper Avenue
Edmonton, Alberta
T5J 3N4

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DEVELOPMENT OF THE *SPIRILLUM VOLUTANS* MOTILITY TEST FOR TOXICITY STUDIES

by

R.L. MOORE

Department of Microbiology and Infectious Disease
University of Calgary

for

Research Management Division

Alberta Environment

March 1982

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ABSTRACT

The *Spirillum volutans* motility test has been recommended as a useful procedure for the assessment of water quality (Bowdre and Krieg 1974). The basis of this test rests on the ability to observe a change in the motility of this bacterium in the presence of toxic substances. The present study was carried out to examine procedures which would increase the sensitivity and usefulness of this test.

It was found that tests performed at 36°C or pH 8.0 showed a significant increase in sensitivity over tests performed at room temperature and pH 6.8, as recommended for the standard test. The test was easily performed with a microperfusion chamber which provides a stable optical platform and the capability of fully automating this test. The potential of developing a multitest system with the basic equipment required for the automation of this test is discussed.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

Pollution of the environment due to intensive agricultural practices and increasing industrialization, together with a better informed and more concerned public, has led to greater research efforts to develop procedures for environmental monitoring in recent years. Because of cost advantages and the ease of application with complex systems, a number of biological systems employing various species of microorganisms have been investigated for this use (Dutka 1978). The present report deals with one of the newer of these procedures, the *Spirillum volutans* motility test.

This bacterium was first described in 1832 by Ehrenberg but was not grown in pure culture until 1962 (Rittenberg and Rittenberg 1962). It is an unusually large bacterium measuring 1.5 to 1.8 μm in diameter and 18 to 40 μm in length. It is microaerophilic and motility is produced by a bundle of flagella located at each of two poles of the cell. The flagellar bundles rotate at about 40 revolutions per second and periodically reverse their direction of rotation (Krieg et al. 1967). This produces a characteristic pattern of motion in these cells referred to as reversing motility in which the cells are seen to rapidly and frequently change their direction of progress. The observation that the coordination of the direction of rotation between the two flagellar tufts could be disrupted by relatively low levels of certain compounds (Krieg et al. 1967; Caraway and Krieg 1972) led to the use of this bacterium for detecting the presence of toxic compounds in fresh waters (Bowdre and Krieg 1974).

The purpose of the present investigation was to examine the potential of adapting the *S. volutans* test for use with a microperfusion growth chamber to provide a means for the eventual automotion of this test, and to see if the sensitivity of the test could be improved beyond the present assay by altering the temperature or pH.

2. MATERIALS AND METHODS

2.1 BACTERIA AND MEDIA

The organism used throughout this study, *S. volutans* (ATCC 19554), was kindly supplied by Anser Qureshi, Alberta Environmental Centre. The organism was maintained on the medium described in Table 1. This medium differed from the original medium of Bowdre and Krieg (1974) in that it lacked $MnSO_4$, contained only half the concentration of $MgSO_4$, contained KH_2PO_4, and Bacto-Casitone was substituted for the salt-free, vitamin-free casein hydrolysate. Better growth was obtained with a medium containing a low concentration of agar rather than with broth alone, and at 30°C rather than at room temperature.

The cell density of the cultures employed in growth experiments was determined by measuring their absorbance with a Beckman Acta II Spectrophotometer.

2.2 STANDARD TEST

The procedure used for the *S. volutans* motility test was that described in the preliminary report of task group D, ASTM Committee 19:24 on water, B. Selconis, Chairman (Table 2). The only change from this procedure was that a reading of the test was also made at 20 min. The formulation for the defined test medium used in this procedure is given in Table 3.

2.3 MODIFIED TEST

In the modified procedure, the test was carried out with the cells contained in a microperfusion chamber rather than in test tubes. The chamber that was used (Moore and Duxbury 1981) was a modification of the one designed by Duxbury (1977). A diagram of the chamber is shown in Figure 1. A cellophane membrane (type 345 OM5, British Sidac, St. Helens, Lancs.) shown in red is held between the upper (yellow) and lower (green) parts of the chamber.

Table 1. Maintenance medium for *S. volutans*[a].

Bacto-Casitone	2.5 g
Sodium succinate·6 H_2O	1.0 g
$MgSO_4 \cdot 7\ H_2O$	0.5 g
$(NH_4)_2SO_4$	1.0 g
$FeCl_3 \cdot 6\ H_2O$	0.002 g
KH_2PO_4	0.5 g
Difco-agar	1.2 g
Distilled water	1000 mL
pH adjusted to 6.8 with KOH	

[a]Adapted from Bowdre and Krieg (1974).

Table 2. Standard test procedure for the *S. volutans* motility assay[a].

1. Remove tubes of 10X Defined Test Medium (DTM) from the refrigerator and allow to reach room temperature (water-bath emmersion helps).
2. Pipette 0.1 mL of the 10X DTM solution into sufficient tubes for sample to be tested and controls. Label tubes.
3. Add 0.8 mL of the test solutions, water, or effluent sample to the above labelled tubes.
4. To the first labelled tube add 0.1 mL of healthy bacteria from overnight culture. Swirl tube once.
5. Do not shake vigorously. Quickly make a drop preparation and examine under microscope. This is '0' hour reading.
6. Prepare fresh drop slide preparations and examine, at 5, 10, 30, 60, and 120 min.
7. After the first examination of 10 min, repeat step (6) with new sample. With practice, the first three samples can be started within 5 min and the next two within the next 5 min. Timing and time-keeping is crucial.
8. If, during any one examination, the reversing motility has been eliminated in more than 90% of the cells, a positive result for toxicity has been obtained.
9. All positive results should be repeated to confirm time required to obtain positive results and, if pure compound, the relationship of one higher and one lower dilution. The minimum effective concentration of a toxicant (pollutant) defined by Bowdre and Krieg is the minimum concentration necessary to eliminate reversing motility in more than 90% of *S. volutans*. If more sensitive readings are needed, a gradient may be established, but not really recommended.

Continued ...

[a]Source: Preliminary report of task group D, ASTM Committee 19:24 on water, B. Selconis, Chairman.

Table 2. Continued.

0 to 25%	reversing+
25 to 50%	reversing++
50 to 75%	reversing+++
75 to 90%	reversing++++
>90%	reversing 5+

10. To judge the number of cells having lost the reversing and motility, scan the whole drop, being sure to check different depths within the drop. If this procedure is not followed, false positive results may occur. The whole procedure, from drop preparation to full examination, should take between 30 and 45 sec.
11. Because the decision (whether 90% or more of the *S. volutans* are uncoordinated) is a judgement by the observer, it is recommended that positive controls be run using known toxicants such as Zn^{2+}, Cu^{2++}, Ni^{2+}, or Pb^{+} at concentrations ranging from .001 to .1 mg per 0.8 mL. To confirm that uncoordinated movements are the result of the sample or toxicant, a negative control using 0.8 mL of distilled water should be included in test procedure. From these positive and negative controls, the nature of a positive reaction (90% uncoordination) will soon be apparent, and only rarely will the observer have difficulty when the uncoordinated level is between 80 and 90%.
12. Photograph through the microscope, using film speed ASA 1000, can be made to illustrate positive and negative results. It has not been found necessary to sterilize water or effluent samples, as the contained microorganisms do not appear to interfere with the test.

Continued ...

Table 2. Concluded.

13.	If, however, the sample is heavily contaminated so that the *Spirillum* are prevented from freely swimming about, then a sterilization procedure must be used. Sterilization may be accomplished by membrane filtration or a brief exposure in a microwave oven. Brief microwave exposure for 10 mL in a test tube is 3 10-s bursts of full power in a 750-W microwave oven. Period between power bursts is 1 to 2 s.

Table 3. Defined test medium (DTM)[a].

$MgSO_4 \cdot 7H_2O$	0.5 g
$(NH_4)_2 \cdot SO_4$	10.0 g
Ethylenedimine Tetra-Acetic Acid (EDTA)	0.073 g
N,N-Bis-2-Hydroxyethyl-2-Aminoethane Sulfonic Acid (BES buffer)	2.133 g
Double glass distilled or low resolution water	1.0 L

This preparation is 10X.

Adjust pH to 6.8 with KOH after dissolving all ingredients.

Dispense into screw capped 16 X 150 mL tubes in 10 mL aliquots.

Sterilize by autoclaving in 116°C for 20 min.

Store tubes in dark at 4°C.

[a]Source: Bowdre and Krieg (1974).

Figure 1. A schematic drawing of the microperfusion chamber.

The upper part contains a chamber for the water from a water bath used for temperature control and a vacuum line for connection to a vacuum pump which secures the membrane against a nitrile rubber O = ring (black). The lower part of the chamber has a circular microscope slide coverslip (red) to allow the passage of light and the medium entry and exit tubes (black).

Excess gases were removed from the solution to be tested by a short, 2 to 3 min treatment under vacuum (approximately -1 atm) in order to prevent the build-up of small bubbles which may interfere with the system. The solution was then placed in a plastic 500-mL bag (JB-03-03, Baxter Travenol Laboratories, Malton, Ont.) which was connected to the entry tube by means of a hospital administration tubing set (JC002, Baxter Travenol Laboratories). The flow rate was adjusted to approximately 1 mL/min.

Cells from a 48-h old culture were diluted about 1:10 with distilled water to give a final concentration of approximately 5×10^7 cells/mL. Two drops of the cell suspension were placed on the cellophane membrane and covered with a 13 mm diameter circular glass coverslip (black). The time of coverslip placement was recorded as time zero.

Cell motility was observed with a Zeiss Photomicroscope II fitted with a Panasonic 1050 camera with a Newvicon tube. The camera was connected to a Panasonic Time-Date Generator, Model WJ-800, a Panasonic Time Lapse Video Tape Recorder, Model NV 8030, and a Sony Triniton 17 inch monitor. Figures 2 and 3 show this equipment in use during a test. Not shown is a small portable hairdryer which was used at the low setting during long tests to prevent the buildup of condensation on the objective lens.

A Vicon Motion Detector, Model V222MD, (Vicon Industries, Plainview, N.Y.) was used in all experiments which required a motion detector.

A Videoprint Model 5000 (Image Resource Corp. Westlake Village, Calif.) was used to prepare photomicrographs directly from the videotape signal.

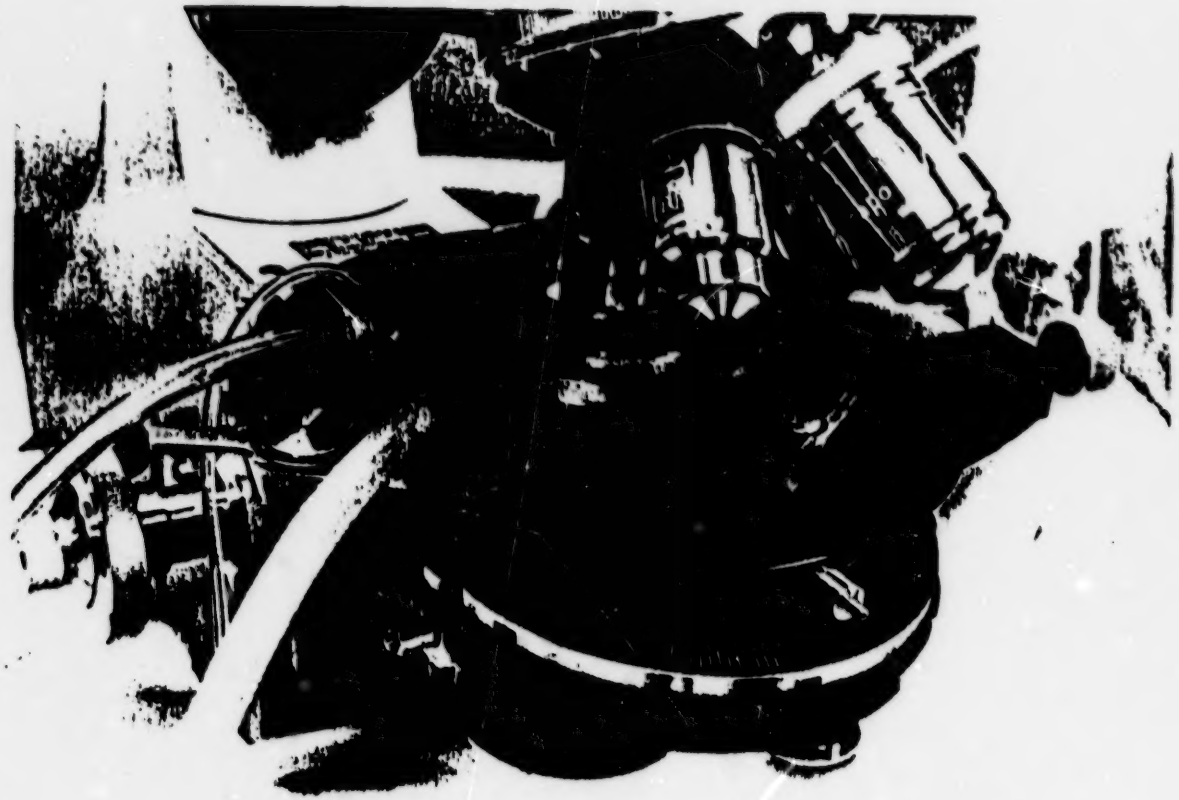

Figure 2. The microperfusion chamber in use.

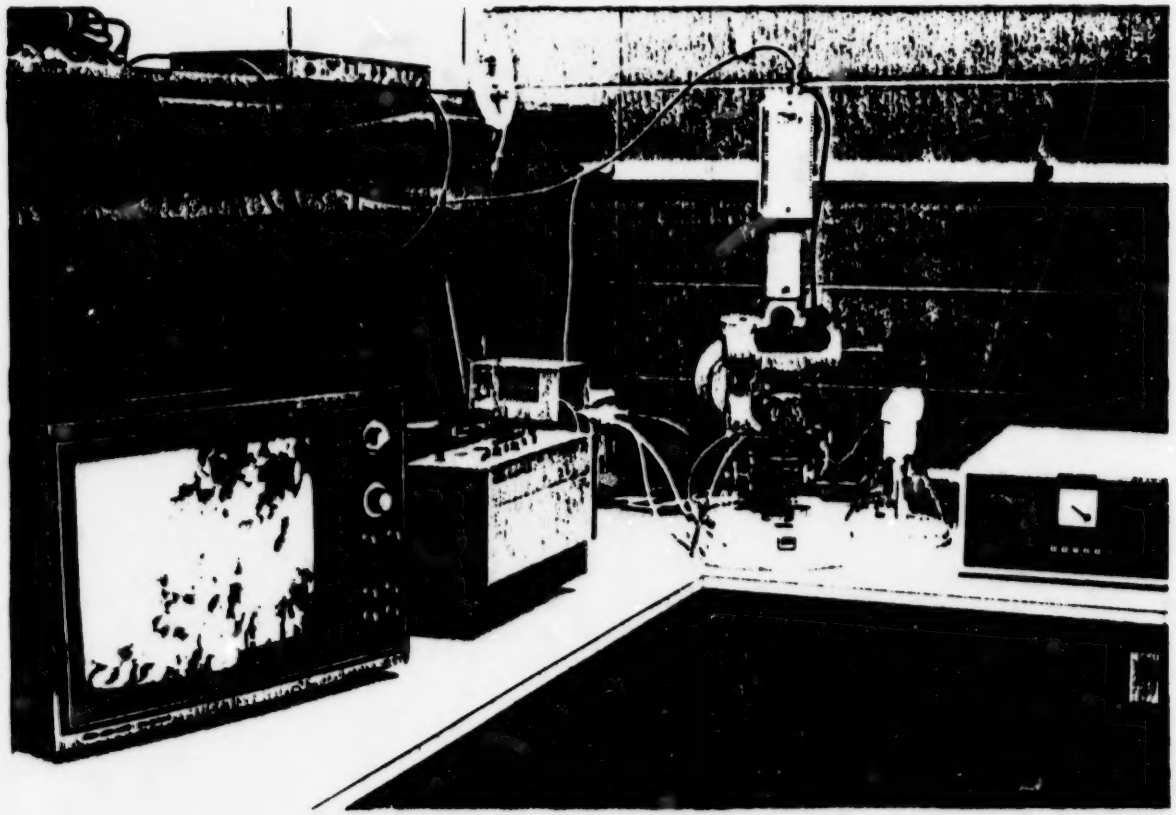

Figure 3. The equipment set up for testing.

3. RESULTS

3.1 EFFECT OF TEMPERATURE

The growth of *S. volutans* at various temperatures is shown in Table 4. Only a small decrease in growth was observed as the temperature was increased from 32° to 36°C. The greatest reduction occurred between 36° and 37°C, although even at 39°C the growth was still slightly greater than at 22°C. The motion of the cells was much more rapid at 36° and 39° than at either 22° or 30°. However, nearly 100% of the cells at 36° were motile while less than half of the cells were motile at 39°C.

Since 36°C was the highest temperature at which nearly optimal growth and motility occur, results with the standard assay procedure at this temperature and the recommended temperature of 22°C were compared (Table 5). The times observed for the cessation of reversing motility in the presence of $HgCl_2$ and sodium lauryl sulphate (SLS) at the elevated temperature were reduced considerably. No difference in the time of inhibition was observed at the concentration of phenol which was selected.

These results were easily reproduced from test to test and this remained so even if the inoculum which was used came from cultures which ranged in age from 24 to 72 h. Preliminary results suggested, however, that inoculum size does effect reproducibility, although this aspect was not pursued further in the present study.

3.2 EFFECT OF NUTRIENT CONCENTRATION

The growth of *S. volutans* of the maintenance medium used in these studies was as good or better than the growth obtained in the maintenance medium described by Bowdre and Krieg (1974). When the cells were transferred from the maintenance medium and mixed with distilled water, their movement appeared much more active than when they were observed in undiluted maintenance medium alone.

Table 4. The effect of temperature on the growth of *S. volutans*[a].

°C	Abs.$_{540}$ nm
22	0.077
32	0.182
33	0.177
34	0.168
35	0.151
36	0.166
37	0.099
39	0.087

[a]Values are the average of three tests after 24-h growth in maintenance medium of pH 6.8 lacking agar. Inoculum is from a 24-h culture incubated at 30°C. Absorbance of starting cultures was 0.040.

Table 5. *S. volutans* motility test results on three inhibitors.

Cmpd[a]	Conc. (µg/mL)	°C	Min	No. Tests
$HgCl_2$	10	22	30	10
$HgCl_2$	10	36	20	10
SLS	80	22	10	10
SLS	80	36	5	10
phenol	800	22	20	10
phenol	800	36	20	10

[a]Phenol and $HgCl_2$ were from Fisher Scientific Co., Springfield, Mo., and SLS was from Sigma Chemical Co., St. Louis, Mo.

While this is partly due to the dilution of the agar which is present in the maintenance medium, it might also result from the dilution of other constituents of the medium. To test this possibility, 1:100 dilutions of maintenance medium cultures and distilled water were prepared and allowed to incubate at temperatures of 22°, 30°, and 37°C. In each case, the motility of the cells after 3 h of incubation was indistinguishable from that of freshly prepared dilutions of maintenance cultures at the respective temperatures. This suggests that the agar was the only ingredient of the medium which was interfering with cell motility.

It was also observed that the motility of the cells in distilled water continued for at least 12 h at 22° or 30°C and 5 h at 37°C. Thus, distilled water could be substituted for growth medium in the microperfusion chamber employed in the modified assay without an adverse effect on the motility of the cells during the expected time interval required to carry out a test.

3.3 EFFECT OF pH

The growth of *S. volutans* at various pH's is shown in Table 6. The poorest growth over the range examined was obtained at 6.2 and 6.8. The latter value is the recommended pH for the standard assay. The best growth occurred at pH 8.0.

Since various inhibitors would be expected to exhibit different pH optima in their interference with motility, the activity of the three inhibitors examined in this study were compared at pH 6.0, 7.8, and 8.0 (Tables 7 and 8). This comparison was made with the modified *S. volutans* motility test described in Section 2. On the basis of the above results, a temperature of 36°C was employed and the medium fed to the microperfusion chamber was a solution of 0.01 M BES adjusted to the desired pH, and one of the test compounds. Controls were performed with this buffer solution without the test compound. The appearance of the cells is demonstrated in Figure 4.

Table 6. The effect of pH on the growth of *S. volutans*[a].

pH	n	$\bar{x}$	s
6.0	3	0.112	0.004
6.2	9	0.087	0.014
6.4	9	0.095	0.014
6.6	9	0.091	0.023
6.8	9	0.089	0.027
7.0	9	0.105	0.011
7.2	9	0.120	0.015
7.4	9	0.125	0.021
7.6	9	0.128	0.010
7.8	8	0.127	0.010
8.0	3	0.131	0.002
8.2	3	0.118	0.023

[a] $\bar{x}$ = mean absorbance at λ = 540 nm after 24-h growth at 30°C in maintenance medium lacking agar.
Absorbance of starting cultures was 0.042; n = number of tests; s = standard deviation.

Table 7. Activity of various inhibitors in the modified *Spirillum volutans* motility test.

Inhibitor	Conc. (µg/mL)	pH	Reversing Motility[a] Time (min)	s[c]	Forward Motility[b] Time (min)	s[c]	Number of Tests
$HgCl_2$	10	6.8	6.08	1.06	7.82	2.49	9
$HgCl_2$	10	6.0	4.24	0.69	4.68	0.83	10
$HgCl_2$	10	8.0	2.66	0.39	2.82	0.37	10
SLS	80	6.8	>60	-	>60	-	2
phenol	800	6.8	>60	-	>60	-	2

[a]Length of time required for inhibition of reversing motility by 90% of the cell population at 36°C,
[b]Length of time required to eliminate all forward progress of all cells.
[c]Standard deviation.

Table 8. Activity of two inhibitors in the modified *Spirillum volutans* motility test with polycarbonate membranes.

Inhibitor	Conc. (µg/mL)	pH	Reversing Motility[a] Time (min)	s[c]	Forward Motility[b] Time (min)	s[c]	Number of Tests
phenol	800	6.8	19.67	1.53	34.00	1.00	3
phenol	800	6.0	16.00	1.00	33.67	1.15	3
phenol	800	8.0	6.85	0.35	11.00	0.85	13
SLS	80	6.8	>60	-	>60	-	3

[a]Length of time required for inhibition of reversing motility by 90% of the cell population at 22°C.
[b]Length of time required to eliminate all forward progress of all cells.
[c]Standard deviation.

Figure 4. Control culture after 24 min 51 sec. Darkfield microscopy. Magnification is 363X.

The time required for the inhibition of reversing motility by $HgCl_2$ in the modified test (Table 7) was reduced by more than 50% of the value obtained in the standard test (Table 5) at comparable inhibitor concentrations, a temperature of 36°C and a pH of 6.8. Even greater reductions were observed at the two pH extremes examined, with pH 8.0 producing the greatest inhibitor activity.

The membranes normally used with the microperfusion chamber did not give valid results for phenol or SLS (Table 7) and a different type of membrane was required. The selection of an alternate membrane is discussed in the following section (3.4). The time required for the inhibition of forward motility by phenol in the modified test with a polycarbonate membrane is shown in Table 8. The results at pH 6.8 were the same as those observed in the standard test (Table 5). At pH 6.0 the time was reduced only slightly, but at pH 8.0, the time was reduced by nearly one-third.

The time required for 90% inhibition of reversing motility by the test compounds was compared to an end point based on the time at which all of the cells are unable to make any forward progress. In the latter case, the cells may revolve about their long or short axis, but they cannot move one location in the field of view to another. Examination of the values for the two end points in all of the experiments where this comparison was made with $HgCl_2$ (Table 7; Figure 5) showed that the time required for the inhibition of forward progress was only increased by an average of 6% over the time required for the inhibition of reversing motility. Phenol, on the other hand, required roughly double the amount of time to achieve the forward progress end point (Table 8).

The effect of concentration on the time required by $HgCl_2$ to produce the forward progress end point in the modified test at the optimum pH of 8.0 is shown in Figure 5. The data indicate that a concentration of less than 0.2 µg/mL $HgCl_2$ can be detected within the 2-h period of time recommended for the standard test.

3.4 SURVEY OF MEMBRANES

The cellophane membranes normally used with the microperfusion chamber gave unsatisfactory results with the inhibitors SLS and phenol (Table 7). Dialysis membranes from Fisher Scientific Co. (Springfield, N.J., No. 8-667D), Alpkem Corp. (Portland, Ore., No. A-170-0472-02, Type C) and Technicon Instrument Corp. (Terrytown, N.Y., No. 178-3470-02, Type H) were also examined in the modified assay and found to be unsuitable. The membranes were heated in boiling water for 10 min and rinsed in distilled water, but this did not improve their performance. Polycarbonate membranes (0.05 μm pore size, Nuclepore Corp., Pleasanton, Calif.) gave good results with phenol, however, but not with SLS (Table 8). It was thought that undissolved particles might be present in the solution of SLS which was used, but prefiltering of the solution did not improve these results.

3.5 MICROSCOPIC METHODS

The appearance of the cells was examined under transmitted light with brightfield, darkfield, phase contrast and Nomarski differential interference contrast optics. The magnification and field size produced with a 16X objective lens were the most satisfactory, although objectives of ten to twenty power would be equally useful. Darkfield microscopy was the most convenient system for obtaining rapid focus of the microscopic field and gave by far the greatest cell to background contrast. The appearance of the cells with this procedure is shown in Figure 4. Only 30 W of power were required for the 60-W lamp employed for illumination in order to achieve the optimum darkfield effect. With this system, the video signal was sufficiently strong to allow a motion detector to detect even slight forward progress of single cells. Thus, under these conditions with a time-date generator, the motion detector could automatically determine the forward progress end point which in turn could be automatically recorded onto videotape.

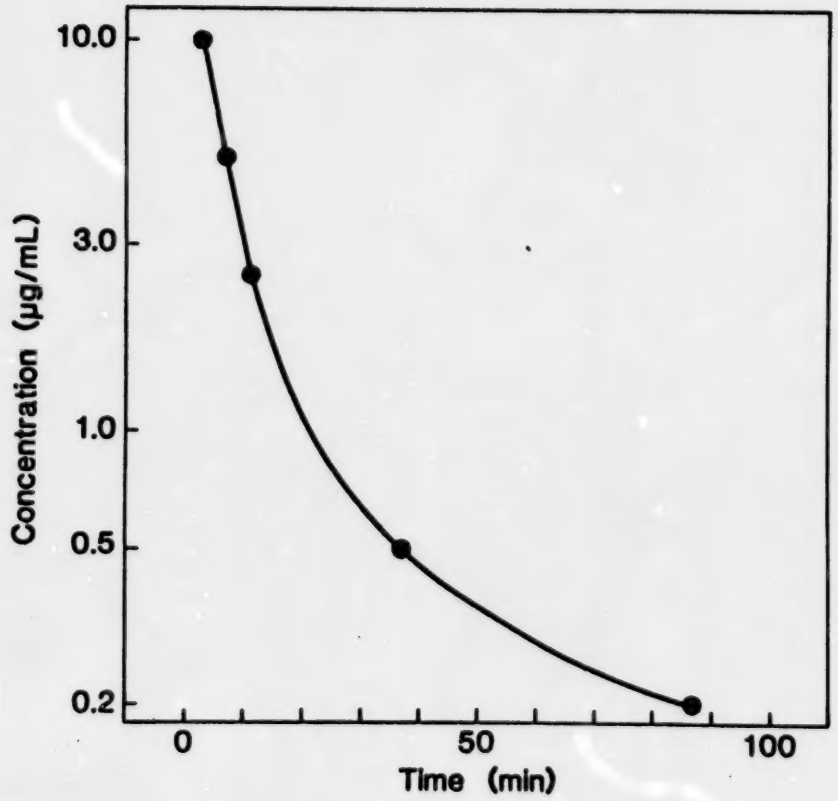

Figure 5. Dose-response curve obtained with $HgCl_2$. Values given are the average of three tests except for 10 µg/mL (10, from Table 7) and 2.5 µg/mL (7). Test solution contained 0.01 M BES, pH 8, and was held at 36°C.

Since any adverse effect on *S. volutans* by the illumination required to visualize the cells would prevent the automation of this test by these procedures, the effect of light on the cells was observed during the course of all the experiments carried out in this project. No tendency for the cells to leave the field of view was ever observed and the motion of the cells appeared to be unaltered.

Because of their opacity, polycarbonate filters did not allow the use of darkfield microscopy as described above. Brightfield, darkfield, Nomarski differential interference contrast, polarized light and oblique lighting procedures with epi illumination were examined for this purpose. None of these methods were satisfactory. Phase microscopy by transmitted light was found to be the most suitable method when polycarbonate filters were used, but this did not produce as high a contrast as darkfield.

4. DISCUSSION

The values obtained for 10 µg/mL $HgCl_2$, 80 µg/mL SLS, and 800 µg/mL phenol with the standard *S. volutans* motility test were 30, 10, and 20 min, respectively. Since 20-min readings are not normally performed in the standard test, these values would have been recorded as 30-min end points. The results with this procedure were identical among comparable sets of tests. They also agreed favourably with the results of previous work to determine the minimum concentration of these inhibitors which could be detected in this test (Bowdre and Krieg 1974; Qureshi et al. 1980; Dutka and Kwan 1981). In addition, it was found that the sensitivity of the test to $HgCl_2$ and SLS could be increased by raising the temperature at which the procedure was carried out to 36°C. The elevated temperature had little or no effect on the normal growth of this organism.

Caraway and Krieg (1972) found that lowering the pH to 4.4 or raising it to 9.9 brought about an interruption of the normal co-ordination between the "head" and "tail" flagellar bundles. However, Dutka and Kwan (CCIW-IWD unpublished report series, manuscript No. 05-AMD-5-81-BD) found that the *S. volutans* motility test could be performed at pH's of 4.5 and 3.5. The lower of these two values improved the sensitivity of the test to aluminum salts by about 15 times. In the present study it was observed that there was little effect on the growth of *S. volutans* over the range of pH 6.0 to 8.0, except that growth and motility seemed to be slightly increased at pH 8. In tests with the modified *S. volutans* motility test carried out on $HgCl_2$ and phenol in the present study, both pH 6 and 8 gave better sensitivity than pH 6.8. In both cases, the best sensitivity was at the highest pH. In this respect, the *S. volutans* motility test has a definite advantage over the best developed and most sensitive bacteriological water quality assay, the Microtox Test (Beckman Instrument Co., Palo Alto, Calif.) which must be carried out at about 6.7. The *S. volutans* test can be performed at the pH of natural waters or at the optimal pH to give maximum sensitivity to a given compound over a very broad range of pH.

The modified *S. volutans* test was found to be more sensitive to the presence of $HgCl_2$ than the standard test. This may have been due to the use of 0.01 M BES with microperfusion chamber rather than the DTM which is employed in the standard assay. While one or more of the constituents of DTM may produce a synergistic effect on the sensitivity of some substances, it apparently had an antagonistic effect in the case of $HgCl_2$. The results with the modified test suggest that it might be usefully employed with natural water samples rather than medium in the media chamber of the microperfusion chamber without any requirement for additives or adjustment in pH.

Since periodic testing is not required by the operator in the modified test, much longer examination times could be employed without a significant increase in labor costs. Longer periods of examination could detect the accumulative effects of even lower levels of toxic substances than are detectable in the recommended 2-h interval. Under some circumstances, it might be much more convenient to increase the length of the time a sample is examined than to have to subject it to one of the procedures required for its concentration. The use of a slow-speed video recorder and time generator allowed high speed playback of a test at up to 96 times the normal speed to determine the end point of a test. Although it was not done in the present study, the end point could be replayed a number of times in order to increase the precision of the value determined for this somewhat arbitrary end point. In any event, the accuracy of the modified test is inherently greater than that of the standard test because the modified test is monitored continuously rather than periodically. The distribution of values obtained with a given test was well within acceptable limits.

The cellophane membranes normally used with the microperfusion chamber in the modified test were found to be inadequate for the detection of SLS or phenol. This was unexpected because this system had previously been shown to allow the passage of the nutrients from media to provide for the growth of *Arthrobacter globiformis* (Duxbury 1977) or *Hyphomicrobium vulgare* (Moore and

Duxbury 1981). The ability of penicillin or mitomycin C to penetrate the membrane had also been demonstrated in these studies. It seems likely that both SLS and phenol could pass the membrane, but at a rate which was too low to produce the desired effect. Further examination of this problem is required. With the large variety of membrane materials presently available commercially, a more suitable membrane can probably be found for use with the microperfusion chamber as it is employed in the modified test. Polycarbonate filters gave adequate results with phenol and this material may have promise for use in future studies.

Motion detectors are normally used in surveillance work to detect motion which occurs within the field of view of a video camera. In order to function properly, the object in motion must make up a significant portion of the field of view and have sufficient contrast to the background. As employed in the modified test, the object size could be regulated by adjustment of the magnification of the microscope and contrast could be adjusted by selection of the type of microscopy used and the intensity of the illumination. The motion detector can be used to signal the time at which motion is first detected or when motion ceases. For this reason a comparison was made in the present study to determine the relationship between the time of inhibition observed in reversing motility and in forward progress of the organisms which was produced by the inhibitors. An increase in time of only 6% was required to reach the forward progress end point with $HgCl_2$, but phenol required about twice the length of time under the conditions at which these experiments were performed. The forward progress end point could be detected by the motion detector, and although the times were longer than observed when reversing motility, this method might still be preferred because no further time is required in addition to the original setting up of the test by the operator and the total length of the test under this system is less important. A more precise system could also be developed, however. With the use of a microprocessor interfaced with the motion detector, the number of motion events detected per unit time could

be recorded. This would allow a direct and continuous comparison with control cultures and in this system the reversing motility end point would be as useful as the forward progress end point.

Since *S. volutans* would maintain its normal motility for prolonged periods of time in natural waters (or could be made to do so with the addition of very modest concentrations of the appropriate nutrients), an automated procedure of the modified test would seem to be most usefully applied to long-term monitoring of natural waters where the presence of inhibitory concentrations of toxic chemicals would occur only sporadically. Under these conditions, it would be particularly useful to have a system which could be capable of adaptation to a remote monitoring system. In addition, this system could be adapted to any test which is based upon the motion of organisms. This would include such tests as the *Daphnia* toxicity test (Buikema et al. 1980) as well as other bacteriological tests. The instrumentation could be easily assembled to perform several of these tests simultaneously and could provide the basis of an automated multitest system. A given biological test system may be more sensitive to a given toxic substance than other biological systems, but no single biological test system has been found to be more sensitive to all toxic substances. For this reason a multitest system has been recommended as the best current approach to the monitoring of water quality (Dutka and Kwan 1981, CCIW-IWD unpublished report series, manuscript No. 05-AMD-5-81-BD; Qureshi et al. 1980).

5. CONCLUSIONS

The sensitivity of the *S. volutans* motility test was increased by raising the temperature from 22° to 36° and by performing the test at one of the two pH extremes, pH 6.0 or pH 8.0.

The test was readily adapted for use with a microperfusion chamber. The results with this procedure were easily reproduced from test to test and were more accurate than those of the standard test because motility was monitored continually. Greater sensitivity with this procedure was also observed.

The microperfusion chamber functioned as an extremely stable optical platform and would provide the means by which the *S. volutans* motility test, and perhaps other biological tests which require the observation of motion, could be completely automated.

6. RECOMMENDATIONS

The following recommendations can be made on the basis of the results of this study: (1) Increase the temperature of the *S. volutans* motility test to 36°C; and (2) Carry out the test at both pH 6.0 and pH 8.0.

Further work should be carried out on the automation of this test. This should include the following: (1) Additional studies on various membranes to determine the most appropriate type or combination of types for this test; (2) Modification of the microperfusion chamber to reduce its construction costs and increase the convenience of its use in routine studies; (3) Expansion of the equipment used in this study to include automatic data collection and recording capabilities for on-line monitoring; (4) Expansion of this system to include the capacity for performing other biological tests simultaneously; and (5) A survey of known compounds, various known combinations of compounds and natural samples with the multitest automated procedure for cost and efficacy comparisons to other procedures for water quality monitoring.

The results of the present study indicate that the *S. volutans* motility test is a reliable test which should be useful in water quality monitoring.

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